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The Viability Of Radish Seeds As  
Affected By High Temperatures And Water Content.



THE VIABILITY OF RADISH SEEDS AS AFFECTED  
BY HIGH TEMPERATURES AND  
WATER CONTENT

BY

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Harry Dwight Waggoner

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BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Doctor of Philosophy

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## THE VIABILITY OF RADISH SEEDS AS AFFECTED BY HIGH TEMPERATURE AND WATER CONTENT.

### INTRODUCTION.

The first important work on the effect of high temperatures upon seeds was done by Edwards and Colin in 1834. From this date to approximately 1880 much interest was shown along these lines, and it is to the activity of the workers of this period that we owe a large and exceedingly valuable literature. The work of Heiden, Sachs, Fiedler, Pouchet, Krasan, Just, Velten, Haberlandt, Nobbe, Höhnel, Detmer and others belongs here. Since 1880 botanical literature contains fewer articles bearing directly on this subject although much work has been done upon the resistance of mosses, molds, and bacteria to heat, and upon the vitality of seeds in general. The more important contributions of this period bearing more or less directly on the viability of seeds are those by Kellermann, Jodin, Dixon, Schubert, Schneider-Orelli and Neuberger.

A full discussion of this rather extensive literature is deemed unnecessary at this time and for it the reader is referred to the excellent work of Just (1877) and Detmer (1880). The more important conclusions reached by former investigators may be summarized as follows:

Seeds lose their viability after exposure to temperatures ranging from 60° C. to 125° C. - Heiden 1859,



Sachs-Fiedler 1865, Haberlandt 1877, Höhnel 1877, Just 1877, Detmer 1880, Neuberger 1914.

Dry seeds are more resistant to high temperatures than those of the same species containing a large amount of moisture,-Haberlandt 1877, Jodin 1899, Dixon 1902.

The injury done to seeds subjected to temperatures approaching the death point increases as the period of exposure is prolonged,-Edwards and Colin 1834, Dixon 1902, Newberger 1914.

Many kinds of seeds may be dessicated in ovens at  $100^{\circ}\text{C}$ . or over for considerable periods of time without apparent injury,-Krasan 1873, Dixon 1902.

Seeds with coats impermeable to water, such as Medicago, can withstand immersion in boiling water for hours without apparent injury,-Pouchet 1866, Nobbe 1876, Dixon 1901, Schneider-Orelli 1909-1910.

There is no definite maximum for the seeds of a given species. Individuals of the same species vary considerably in their resistance,- Just 1877, Höhnel 1877.

The percent of germination of seeds decreases as the temperature to which they are subjected is raised,- Höhnel 1877.

In most instances seeds subjected to temperatures near their death point germinate more slowly and produce less vigorous seedlings than untreated seeds of the same kind.-Krasan 1873, Velten 1876, Dixon 1902. Occasionally the germination of seeds is hastened by exposure to a tempera-



ture a few degrees below their death point, - Haberlandt  
1863, Velten 1876.

Seeds injured by high temperatures become very susceptible to "mold growth," - Dixon 1902.

Seeds injured in treatment show abnormal development such as curled radicle and poorly developed seed leaves, - Dixon 1902.

Seeds immersed in hot water are injured more when oxygen is abundant than when it is lacking, - Haberlandt 1863, Weisner 1871, Just 1877.

A close study of the results obtained by earlier workers shows a wide difference in the maximum temperatures that the same or nearly related seeds can endure without injury. It further reveals the interesting fact that an intimate relation exists between the method employed in treating the seeds and the temperature at which serious injury or death occurs. Briefly, the methods heretofore employed are as follows:

1. The seeds were heated in water or in an atmosphere saturated with moisture. By this mode of treatment it is apparent that the seeds absorbed more or less water during the heating process. Authors using this method invariably report a low lethal temperature for the seeds used.
2. The seeds were heated in small closed containers. By this method the seeds during the process of heating gave up moisture to a greater or less extent until an equilibrium between the seeds and the inclosed air was established.



The results obtained by this method varied according to the amount of water present in the seeds when heated. 3. The seeds were heated in ovens. In this case it is evident that the air in the ovens was of low relative humidity and consequently the seeds lost moisture during the heating. Seed treated in this manner endure exceedingly high temperatures without apparent injury.

It seemed desirable to make a detailed study of the resistance of seeds of different water contents to high temperatures under carefully controlled conditions. Accordingly, experiments were carried on in the laboratories of Plant Physiology under the direction of Prof. Chas. F. Hottes to whom the writer is greatly indebted for searching criticism and helpful suggestion.

In taking up this subject anew two closely related lines of experimentation were outlined. The one was concerned with the effect of high temperature upon series of samples of seeds of increasing water content. The other was to determine the cause for the wide difference in the resistance of seeds treated by the three methods indicated above. A survey of the pertinent literature will serve to bring these two points definitely before us.

As early as 1859, Heiden reported that grains of barley, when exposed for one hour to dry air at 90° C. germinated, while similar grains heated in water at 60° C. for the same period of time were killed. In 1865, Fiedler, working with seeds of pea, rye, flax, barley and corn, showed that



swollen seeds were killed at from  $50^{\circ}$  C. to  $60^{\circ}$  C., while those containing less moisture withstood  $70^{\circ}$  C. or more. The seeds were treated in closed test tubes immersed in water maintained at the desired temperature. Von Höhnel (1877) improved the method as used by Fiedler in that he covered the seeds in the test tubes with fine metal filings to facilitate the transfer of heat. He worked with seeds of a number of different species and reported that most of them when sufficiently dried were able to endure an exposure at  $110^{\circ}$  C. for sixty minutes. Some heated at  $125^{\circ}$  C. for fifteen minutes were found viable. Just (1875, 1877) reports that clover seeds heated in a saturated atmosphere at  $50^{\circ}$  C. for forty eight hours, or at  $75^{\circ}$  C. for one hour, lost their viability. However, if the seeds were well dried they could endure a temperature of  $120^{\circ}$  C. Other kinds of seeds gave similar results. Detmer (1880) records that the viability of seeds is not lost in boiling water provided they are not in a swollen state. He further states that the less moisture seeds contain at the time of treatment the greater is their resistance to high temperatures. Other investigators of this early period obtained similar results.

More recently, Jordin (1899) found that seeds of peas and cress when dried at  $60^{\circ}$  C. for twenty-four hours can be heated at  $98^{\circ}$  C. in the dry air of sealed tubes for six hours and still retain their viability; similar seeds heated in humid air at  $40^{\circ}$  C. lost their viability in twenty hours. In subsequent experiments he found that the resistance of



the seeds of pea and cress was increased in a marked degree when calcium chloride was introduced into the tube with the seeds. He states that seeds may be exposed to dry air at 65° C. for prolonged periods without loss of vitality, but adds that this may be done only if one heats them in an open dish to permit a rapid loss of water from the seeds. If the air becomes saturated with water vapor, the seeds can endure only a comparatively low temperature without injury. Dixon (1902) dried various kinds of seeds over sulphuric acid and later in an oven at 95° C. for several days without destroying their viability. He treated the samples in closed test tubes and found that when sufficiently dry the seeds could withstand temperatures far above that of boiling water (110° C.-120° C.) without injury. Neuberger (1914) and others of recent date, in their studies on the comparative resistance of moist and dry seeds to high temperatures, in a general way, confirm the results of the earlier authors.

Pouchet (1866) working with seeds of *Medicago* obtained from sheep-wool brought from South America, found that they germinated after being exposed to boiling water for four hours. He subsequently experimented with other *Medicago* seeds and found that only those germinated which, after a prolonged treatment in boiling water, did not swell. Nobbe (1876) confirmed Pouchet's results. Dixon (1901) and Schneider-Orelli (1909, 1910) attributed the high resistance of these seeds to the fact that many of the seed coats are impermeable to water.



From the above review, it is apparent that considerable work has been done on the resistance of seeds to high temperatures. The experiments have been carried on with seeds containing widely different and undetermined amounts of water. So far as the writer has been able to determine no one has attempted to study in series the resistance of the same kind of seeds containing definite and known quantities of water at the time of heating. It is only through a quantitative study of this kind that a definite knowledge of the various factors involved can be obtained, and the variation in results harmonized. The problems before us then are: 1, to determine the definite relation between the water content of seeds exposed to high temperatures and their viability, and 2, to explain the different degrees of resistance of seeds exposed to high temperatures when treated by different methods.

#### MATERIALS AND METHODS.

The seeds used in these experiments were Icicle, Black Spanish Winter and Crystal Forcing radish obtained from Henry A. Dreer, Philadelphia. These were selected with the view of comparing the relative resistance of varieties adapted to widely different cultural conditions. The seeds were of good quality and throughout the experiments tested at approximately 90%. They were stored in glass-stoppered bottles and kept in a room where the humidity of the air varied but little; the moisture (4%) in the seeds remained practically constant throughout the experiments. A tin measure was used to obtain



random samples from the bottles and no selection was made other than that the seeds with ruptured coats were discarded.

Three methods were used in heating the seeds.

1. The samples were placed in Florence flasks of 100 cc capacity, the bottoms of which were sufficiently large to allow each seed to come in direct contact with the glass. The flasks were placed in a wire cage and entirely submerged in a bath containing about thirty-five liters of water previously heated to the desired temperature. This large quantity of water, kept in circulation by a Köhler stirrer, rendered it possible to maintain a temperature constant to within one-half of a degree Centigrade. Increase in pressure during the heating was guarded against by providing the containers with corks through which capillary tubes thirty centimeters long were passed. Temperatures above the boiling point of water were obtained by adding the requisite amount of calcium chloride to the water of the bath. In these latter experiments provision was made for replacing the water evaporated from the bath while the experiments were in progress. 2. The samples in shallow open pans were placed in a double walled copper oven filled with glycerine. The temperature was controlled to within one-half of one degree Centigrade. 3. The samples were enclosed in muslin and immersed directly in the water of a bath previously heated to the desired temperature.

The water content of the stored seeds was determined by finding the loss in weight of a random sample brought to constant weight in an oven maintained at  $104^{\circ}$  C. In the ex-



periments requiring a reduction of the water content of air dry seeds, the amount of water present was diminished by heating, first in an oven at 60° C. and later in one at 100° C. Samples were treated when upon weighing, it was found that the water content of the seeds had been reduced to the desired amount. To increase the water content the air dry seeds were exposed to a saturated atmosphere until they had absorbed the amount desired in a particular experiment. This method served very well when only a slight increase in moisture was desired. When a considerable increase was necessary, the air dry seeds were soaked in tap water at 20° C. until they had absorbed the required amount. The seeds, superficially dried between towels, were placed in the flasks and allowed to stand for a time to permit the water to penetrate uniformly before heating. A momentary immersion of the dry seeds in 95% alcohol was found beneficial in that it allowed a quick and uniform wetting of the coats when the seeds were placed in water.

The seeds were germinated on plaster of Paris blocks twelve centimeters square and three centimeters thick. The surfaces of these blocks were crosschanneled so that there were one hundred intersections suitable for the hundred seeds used in each test. The blocks containing the seeds were placed in fiber tubs and water added to a depth of two centimeters. The tubs were then covered and kept in a dark room at approximately 23° C. A daily record of the number of germinated seeds was made, and this continued for fourteen days.



## EXPERIMENTS AND DISCUSSION.

All the earlier investigations in this field were carried on with very dissimilar seeds of widely different, and in no case definitely determined, water content. In no instance was an attempt made to find the effect of a definite high temperature upon a series of like seeds of different but known water contents.

Radish seeds (Icicle, Black Spanish Winter, and Crystal Forcing) with an initial water content of from 4% to 71% as indicated in tables I, II and III, were placed in the flasks already described and heated for thirty minutes at the temperatures ( $50^{\circ}$  C. to  $125^{\circ}$  C.) indicated at the head of the tables. After heating for this period they were placed on the blocks for germination. The data in the tables that follow is that obtained from the treatment of approximately sixty thousand radish seed. The resistance of the three varieties proved to be so similar that it is not necessary to discuss each table separately.







An examination of these tables shows that there is a definite relation between the initial water content of seeds heated at high temperatures and their viability. Seeds of an initial water content of 71%, 50% and 45% are killed at 60° C. As the water content decreases in the successive series we find the percent of germination to increase and the death point of the sample to be markedly higher. For example as the water content is decreased from 45% to 30% the lethal temperature shifts from 60° C. to 65° C. Air dry seeds of approximately 4% water content give a normal germination after treating at 75° C. and are killed between 95° C. and 100° C. On the other hand, samples carefully dried until only .4% of water is present at the time of treatment, give a normal germination at 100° C. and are killed between 123° C. and 125° C. We find then as the water content increases from .4% to 71% that the maximum temperature at which a normal percent of germination takes place drops from 100° C. to below 50° C. and that the lethal temperature drops from between 123° C.-125° C. to 55° C.-60° C. The resistance of radish seeds exposed to high temperatures decreases as their initial water content increases. Furthermore at temperatures high enough to be injurious, the viability of radish seeds of a definite initial water content, decreases as the temperature to which they are exposed is raised.

In recording the percent of daily germination not included in the above tables, a greater or less degree of retardation in the germination of seeds treated at temperatures



a few degrees below their maximum, occurred so constantly that it was thought advisable to make a definite quantitative study of the same. In Table IV are recorded the results of a series of experiments on the rate of germination as affected by initial water content and high temperatures.

Samples of five hundred radish seeds containing 4%, 9%, 14%, and 18% of water respectively, were heated for thirty minutes in 100 cc flasks submerged in a bath held at 80° C. and subsequently allowed to germinate on plaster of Paris blocks. The data (Table I) shows that at this temperature seeds containing the above water contents suffer in direct proportion to the quantity of water present. The observations were carried on over a period of seven days and the results obtained recorded as indicated in Table IV. The daily percent of germination of the five hundred untreated seeds is shown in the first column. In the succeeding columns is recorded the germination of seeds of an initial water content of 4%, 9%, 14%, and 18% respectively, when heated at 80° C. for thirty minutes.

TABLE IV.  
The retardation in germination caused by water content  
and high temperatures.

	Check Untreated	Moisture in seed when heated at 80° C.			
		4%	9%	14%	18%
Percent germinating the 1st day	30	6	.6	0	0
" " 2nd day	45	21	5.4	0	0
" " 3rd day	14	35	17	7.4	0
" " 4th day	3	17	24.2	11	0
" " 5th day	2	3	10	6	0
" " 6th day	1	1	3.6	2.2	0
" " 7th day	0	0	0	.4	0
Total germination	95	83	60.8	27.0	0



The maximum percent of germination in the check (45%) occurred on the second day while in the treated seeds the highest number of seeds germinated on the third or fourth day. Seventy-five percent of the untreated seeds germinated in the first two days as compared with twenty-seven percent for those of 4% moisture, six percent for those of 9% moisture and zero percent for those of 14% moisture. The resulting injury due to the increased water content is shown by the rapid decrease in total germination of the samples, namely: 95%, 83%, 60.8%, 27% and 0% respectively. The retardation in the germination of radish seeds becomes greater as the injury due to the treatment becomes more marked.

In the discussion of the methods employed by earlier investigators in the treatment of seeds to high temperatures, attention was called to the difference in the water content of the treated seeds before and after treatment. To correlate the results found in the literature with my own, it becomes necessary to repeat, under control conditions, the different methods heretofore used. In the preceding section (Table I, II, III.) the intimate relation between initial water content and viability at high temperatures was definitely determined, and the suggestion made that the wide difference in the lethal temperature of seeds of similar kinds as reported by different investigators was to be sought in the initial water content of the seeds or in the method employed that would allow an increase or decrease of this water content during the heating process.



Three series of similar samples of radish seeds containing an initial water content of 19% were heated at temperatures from 45° C. to 105° C. for a period of thirty minutes (Table V). The samples of Series 1 were heated directly in water, those of Series 2 in 100 cc flasks immersed in water, and those of Series 3 in an oven. The treated seeds together with the untreated checks were placed upon the plaster blocks for germination. The results in percent of germination are recorded in Table V.

TABLE V.  
The effect of heating radish seeds with an initial water content of 19%.

	Temperatures at which radish seeds were heated for 30 min.												
	45°	50	55	60	65	70	75	80	85	90	95	100	105
Series 1. Heated in water	38	80	58	2	0	0							
Series 2. Heated in 100 cc flasks													
Series 3. Heated in oven		89	87	81	66	18	9	0	0				
Checks, untreated	88	90	89	88	89	91	90	90	89	88	90	90	89

The results as given in this table show very clearly that radish seeds of similar water content when heated as indicated in Series 1, 2, and 3 respectively exhibit very different degrees of resistance. The samples heated directly in water suffered a loss at 50° C. and were killed at 65° C.; those heated in the flasks suffered a similar loss (approximately 8%) at 60° C. and were killed at 80° C.; those heated in the oven suffered a loss at 85° C. (5%) and were killed at 105° C. Further it is to be noted that in Series 1 the effects of the temperature are distinctly manifested at 50° C. and that from this temperature the viability decreases very rapidly, extending through a range of only 15° C. In Series 2 similar effects are noted



$10^{\circ}$  C. higher, namely  $60^{\circ}$  C. and the viability decreases less rapidly, namely extending through a range of  $20^{\circ}$  C. The most marked effect is shown in Series 3. The effects of the treatment here lie  $35^{\circ}$  C. higher than in Series 1 and  $25^{\circ}$  C. higher than in Series 2. The decrease in viability is slow at first and then very rapid, falling from 60% germination at  $95^{\circ}$  C. to 6% at  $100^{\circ}$  C.

The data recorded in Table V Series 2 is practically a repetition of that found in Tables I, II, and III, for an approximately similar initial water content. Starting with an initial water content of 19% in Series 1 Table V. we find the injury essentially equivalent to that of seeds with an initial water content of approximately 71%, (Tables I., II., III.). In Series 3 Table V. with the same initial water content we find the injury essentially the same as that for seeds of 4% initial water content (Tables I., II., III.). This is exceedingly suggestive.

Three series of weighed samples of radish seeds with an initial water content of 19% were heated at  $65^{\circ}$ C.,  $80^{\circ}$ C. and  $95^{\circ}$  C. for periods of 3, 6, 10, 15, 22, and 30 minutes respectively. The series were repeated for each of the three methods already indicated in Table V. At the end of each period of heating the samples were removed, weighed, and the loss or gain recorded as indicated in Tables VI., VII., and VIII. The data from these tables was used in constructing the graphs in Plate I. The ordinates express the percent of gain or loss in weight of the samples (above or below 19%) according as the graph extends



above or below the line A B. The abscissae represent the periods of time during which the seeds were exposed to the respective temperatures. In order to obtain the actual water content of the seeds, one must add or subtract the indicated gain or loss (Plate I.) to or from the initial water content (19%) of the seeds used.

TABLE VI.

The percent of increase in weight of seeds containing an initial water content of 19% when heated in water.

Temperatures	Time of exposure					
	3 min.	6	10	15	22	30
65°	13%	21	29.4	37	45	53.2
80°	20.1	32.2	45	57.1	60.3	61
95°	23.4	39	51	59.8	60.1	60.4

TABLE VII.

The percent of loss in weight of seeds containing an initial water content of 19% when heated in 100 cc flasks.

Temperature	Time of exposure					
	3 min.	6	10	15	22	30
65°	2%	3	2.9	3	3	2.9
80°	4	4.9	5	5	5.1	5.1
95°	7.3	8	7.9	7.9	8	8

TABLE VIII.

The percent of loss in weight of seeds containing an initial water content of 19% when heated in an oven.

Temperature	Time of exposure					
	3 min.	6	10	15	22	30
65°	3%	5	6.3	7.9	9.8	10.5
80°	4	6.3	8.0	10	12.1	13
95°	5.3	8	10.1	12.4	14.4	15.9



These graphs show the striking changes in water content obtained by heating radish seeds of 19% initial water content by the three different methods. (A subsequent experiment will show that the gain in weight of the samples heated by method 1 does not express accurately the amount of water absorbed). The seeds heated directly in water increased in weight very rapidly; those heated in the flasks decreased in weight for about six minutes and then remained constant during the remainder of the heating; those heated in the oven gradually decreased in weight throughout the entire thirty minute period of treatment. At the end of thirty minutes the seeds heated at 95° C. were found to have a water content for method 1, of 79%, method 2, 11%, and method 3, 3%. A similar variation, though less extensive, is seen in seeds heated at 80° C. or 65° C. respectively (Plate I.) It has been shown that the resistance of seeds exposed to high temperatures is inversely proportional to the initial water content at the time of heating. The results obtained in the above experiments (Plate I. Table V.) are in entire accordance with this statement.

Data are not at hand upon which to base a complete explanation of the resistance of radish seeds to high temperatures. Seeds heated in air lose water by evaporation which is a cooling process. Moreover, air and water differ materially in their ability to transmit heat. Hence we cannot say with absolute certainty that we have subjected the seeds used in the corresponding tests to exactly the same temperatures for the same periods of time. Data from seeds heated at a given tem-



perature without suffering any change in the water content during heating would be desirable. However the results as shown in Table V. between water and oven heated seeds cannot be explained on the basis of cooling. These questions together with related ones will form the basis for a future investigation.

The rate of gain or loss in water content of the seeds when treated by method 1, 2 or 3 is of interest. This is directly related to the low (Series 1) or high (Series 3) resistance of the seeds. The rapid absorption of water in the seeds of Series 1 concomitant with the high temperature is responsible for the marked injury as indicated in Tables I., II., III., and V. On the other hand the rapid loss of water in the first six minutes of treatment in Series 2 and the subsequent maintenance of approximately the same water content for the whole period of heating - due to equilibrium of vapor tension - brings the resistance markedly higher. In the seeds of Series 3 the water vapor was constantly carried off and consequently the seeds lost water throughout the process.

Attention should be called to the fact that radish seeds heated directly in water lose in dry substance. It is necessary to know the extent of this loss in order to interpret more accurately the results found in Table VI. This experiment was repeated and the loss of dry matter determined and recorded as shown in Table IX.



TABLE IX.  
The loss (in percent) in dry weight of radish seeds when heated in water at  $65^{\circ}$  C.,  $80^{\circ}$  C., and  $95^{\circ}$  C. respectively.

Temperature	Time of exposure of seeds					
	3 min.	6	10	15	22	30
$65^{\circ}$	21%	.4	.7	1	1.4	2
$80^{\circ}$	.39	.82	1.4	2.1	3.6	5.4
$95^{\circ}$	.78	1.6	2.6	4	6.1	7.9

These results show that there is a gradual loss in weight when radish seeds are heated directly in water at the temperatures indicated. Moreover the higher the temperature of the water the more rapid is the loss in weight. The seeds heated at  $65^{\circ}$  C.,  $80^{\circ}$  C., and  $95^{\circ}$  C. for thirty minutes lost 2%, 5.4% and 7.9% respectively. It follows from this that the actual amount of water taken up by radish seeds heated directly in water is considerably more than the increase in weight as indicated in Table VI.

#### Conclusions.

The resistance of radish seeds exposed to high temperatures is inversely proportional to the initial water content of the seeds at the time of heating.

At temperatures high enough to be injurious the viability of radish seeds of a given initial water content decreases as the temperature to which they are subjected is raised.

The general resistance of Icicle, Black Spanish Winter, and Crystal Forcing radish seeds exposed to high temperatures is very similar.

Radish seeds injured by water content and high temperatures are retarded in their germination. This retardation



becomes more marked as the temperature or water content or both is increased.

Radish seeds of the same initial water content show very great differences in resistance when heated at the same temperature but by different methods, namely: in water, in flasks or in ovens.

The amount of water absorbed or given off by radish seeds during treatment is the chief factor determining the resistance of the seeds heated at the same temperature by the different methods.

When radish seeds are heated directly in water they suffer a gradual loss of dry substance. This loss becomes greater as the temperature of the water is increased.



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## VITA.

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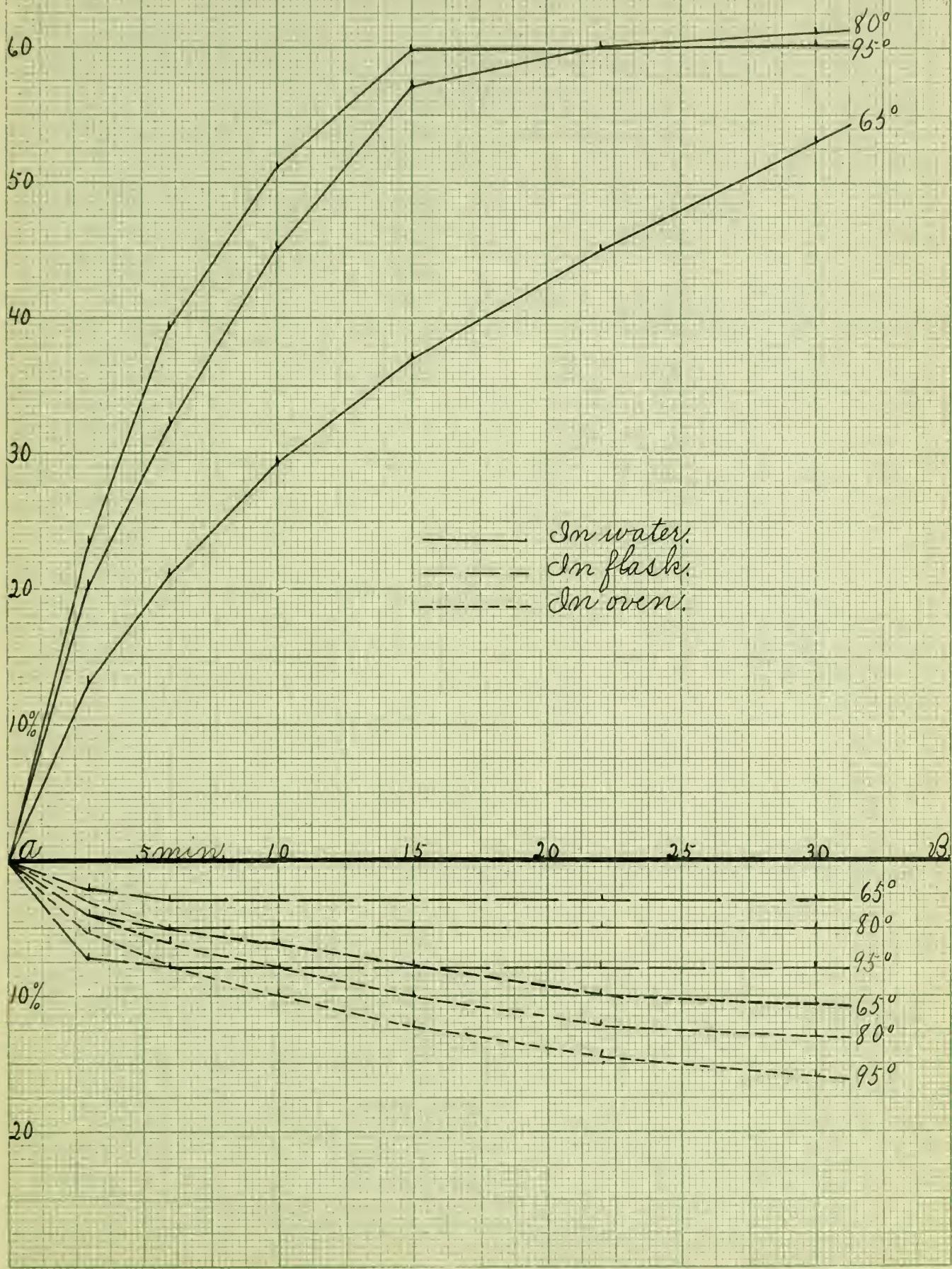
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Plate I.







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